PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 146 337-348 (1974)

Adult and Embryonic Gecko Cells *in Vitro*: Growth Characteristics, Infection by Rabies, Sindbis and Polyoma Viruses, and Transformation by SV40 (38100)

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(Introduced by David Kritchevsky)
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Reptilian cell lines described to date have been derived from normal or tumor tissues of adult animals (1–5). One of these, cell line GL-1 from the lizard *Gekko gecko* has been shown to be susceptible to "transformation" by the simian papovavirus SV40 (6). We describe here the establishment and comparative characterization of cell lines from adults and embryos of the lizard *Eublepharis macularis,* the leopard gecko. Cell lines of this species are of special potential usefulness, since the animals may be bred and reared in captivity (Slemmer, G., unpublished observations). It is possible to cultivate continuously cells from tail tissue removed without damaging the host individual (see below). Transformation by SV40 of these cell lines is reported.

Materials and Methods. Reptiles. Leopard geckos, lizards native to northern India and Pakistan, were generously contributed by Dr. Glen Slemmer of the Institute for Cancer Research, Fox Chase, Pennsylvania. They were bred and reared in captivity.

Initiation of cell lines. The embryo used as a cell source was in the 33rd day of a normal 61–66 day development period of eggs incubated at 27–29°. A 3-cm embryo was washed five times with phosphate buffered saline (PBS) and minced. Portions of minced tissue suspended in Eagle's basal medium containing 10% fetal calf serum (BME-FCS 10), penicillin (200 units/ml) and streptomycin (100 μg/ml) were explanted into plastic (Falcon) 25 cm^2 flasks. Minced tissue was also dispersed by mechanical agitation in trypsin–versene solution (0.25% trypsin and 0.1% versene in Ca^{2+}– and Mg^{2+}–free PBS) at room temperature (22–25°) for 30 min. The digest was filtered through sterile gauze and planted in plastic flasks at a concentration of 1×10^6 cells/ml in BME-FCS 10. Duplicate primary cultures were incubated at 30 and 33°. Superior cell growth was obtained at 30°, which temperature was employed for all further studies.

To initiate adult cell lines, visceral organs and a mass of tail muscle and subcutaneous connective tissue from a 6-mo-old gecko were explanted directly or dispersed and planted at cell concentrations ranging from 6×10^3 to 3×10^5 cells/ml. The methods of cultivation of BHK/21 and African green monkey (CV-1) cells and of reptile cell lines *Terrapene* heart-1 (TH-1), *Gekko* lung-1 (GL-1), and *Iguana* heart-2 (IgH-2) have been reported elsewhere (1, 2, 7, 8).

Morphologic studies. Cells were plated on cover slips in Petri dishes at two to five passage intervals. Confluent monolayers were fixed in 100% methanol and stained by the May–Greenwald–Giemsa technique. Cell size was determined by the use of a Coulter counter particle size distribution plotter, model "H"; cell counts were performed with a hemocytometer. The absolute plating efficiency was determined by plating 1×10^4 cells in 5 ml of growth medium in a 25 cm^2 plastic flask. After 10 days of incubation at 30°, the cultures were stained with crystal violet and colonies of two or more cells were counted with the aid of a microscope.

Karyologic studies. Karyotypic techniques have been previously described (9). Cultures for karyotypic study were pretreated with 0.05 mg/ml of Colcemid, subjected to hypotonic shock (1.0% Na citrate solution), and stained with 2% acetoorcein after the method of Moorhead *et al.* (10) with minor modifications.

Viral studies. Vesicular stomatitis virus (VSV) (Indiana strain), rabies (ERA strain), Sindbis, herpes simplex (HF strain), and vaccinia viruses were propagated in BHK/21 cell culture. Newcastle disease virus (NDV) stocks were prepared in primary chick embryo fibroblast culture. Frog polyhedral virus LT-1 (11) and frog adenovirus FAV-1 (12) were propagated in TH-1 cells. Duplicate cell cultures in tubes were inoculated with 0.1 ml of the undiluted virus stocks listed above (multiplicity of infection [m.o.i.] = 0.10–10.0) and incubated at 37° for the homeothermic vertebrate viruses, or 25 and 30° for the frog viruses LT-1 and FAV-1, respectively. Cultures exhibiting cytopathic effect (CPE) were harvested by three cycles of freezing and thawing, and a second passage was performed using undiluted first passage material. SV40 (RH 911) propagated in CV-1 cells was used for transformation studies. Assay for the SV40 T antigen was performed by the indirect immunofluorescence method (13). Polyoma virus was grown in mouse embryo fibroblasts.

Optimum temperature determination. Cells were seeded at a concentration of 8.0×10^4 cells/ml in 25 cm^2 plastic flasks and allowed to attach for 24 hr at 30°. The flasks were then placed in separate stations of a temperature gradient incubator (14). The incubator maintained temperature within a tolerance of ± 0.2°. The cells in each flask were counted when a confluent cell monolayer was first observed at the optimum temperature.

pH optimum determination. Replicate cell cultures were seeded at concentrations of $4.0–8.0 \times 10^4$ cells/ml in 5 ml of Eagle's minimal essential medium supplemented with 10% FCS and buffered at selected pH levels with organic ions (15) (provided by Dr. Harry Eagle) and incubated at 30°. The number of cells per flask was determined on days 1, 4, and 8. Medium was changed and the pH monitored on days 2 and 5. The pH of these media remained constant (variation = ± 0.2 pH units) throughout the course of the experiments.

Cell colony formation suspended in agarose. A modification of the technique of Macpherson (16) was used: 2.0×10^5 cells suspended in 2 ml of BME-FCS 10 containing 0.3% agarose were plated upon a base layer of 5 ml of 2X BME containing 0.5% agarose in 25 cm^2 plastic flasks and incubated at 30°.

Results. Initiation of cell lines. The cell lines established in this study are listed in Table I. Cell line GE-1 was established from trypsin-dispersed embryonic cells; cell line GE-2 was derived from embryo tissue explants. Cell lines WGT-1 and WGT-2 were similarly established from adult tail fragments (muscle and connective tissue), but cell growth at early passage levels was much less vigorous than that obtained with embryonic tissue. Cell lines were not obtained from adult heart, tongue, spleen, lung, or kidney tissues. Cells from adult lung and kidney tissues were serially cultivated for six passages, but then degenerated after exhibiting highly vacuolated cytoplasm and numerous multinucleated giant cells. Attempts at isolation of virus from these degenerating cells were unsuccessful.

Cell morphology. Both GE-1 and GE-2 cell lines were derived from primary cultures exhibiting a population of mixed cell types. The GE-1 cell line became predominately epithelial-like after approximately five passages (Fig. 1A); GE-2 was predominately fibroblast-like in early passages but became more epithelial-like after about 20 passages. WGT-1 and WGT-2 cell lines originated from predominately fibroblast-like primary cell cultures and have remained predominately fibroblast-like to date (Fig. 1C).

Karyologic studies. The normal diploid karyotype of *E. macularis* was determined from embryo cells in the third passage *in vitro.* The 2N number was 38 and all elements of the karyotype were acrocentric, forming a graded series according to size (Fig. 2). GE-1 cells examined at passage 12, 17, and 25 showed no deviation from the normal diploid karyotype. The incidence of chromatid breaks ranged from 0 to 2% and the level of tetraploidy ranged from 4 to 8%. GE-2 cells examined at the 20th passage level and WGT-1 cells observed at the fifteenth passage also retained the normal diploid karyotype.

Growth rate. The mean population doubling time at 30° of GE-1 cells (passage 23) and of GE-2 cells was about 3.5 days. WGT-1 cells grew very slowly for the first 12 passages (about 14 wk) (mean population doubling

TABLE I. *Eublepharis macularis* Cell Lines.[a]

Cell line		Method of initiation	Months in passage to 1/73	Total passages to 1/73	Cell morphology	Mean population doubling time (days)	Modal chromosome number ($2n = 38$)
Embryo	GE-1	Trypsinization	15	57	E	3.5	38
	GE-2	Explant	15	45	F → E	3.5	38
Adult	WGT-1	Trypsinization	13	45	F	3.5	38
	WGT-2[b]	Explant	10	14	F	ND	ND

[a] Abbreviations: E = epithelial like; F = fibroblast like; ND = not determined.

[b] Lost at passage 14 because of failure to grow.

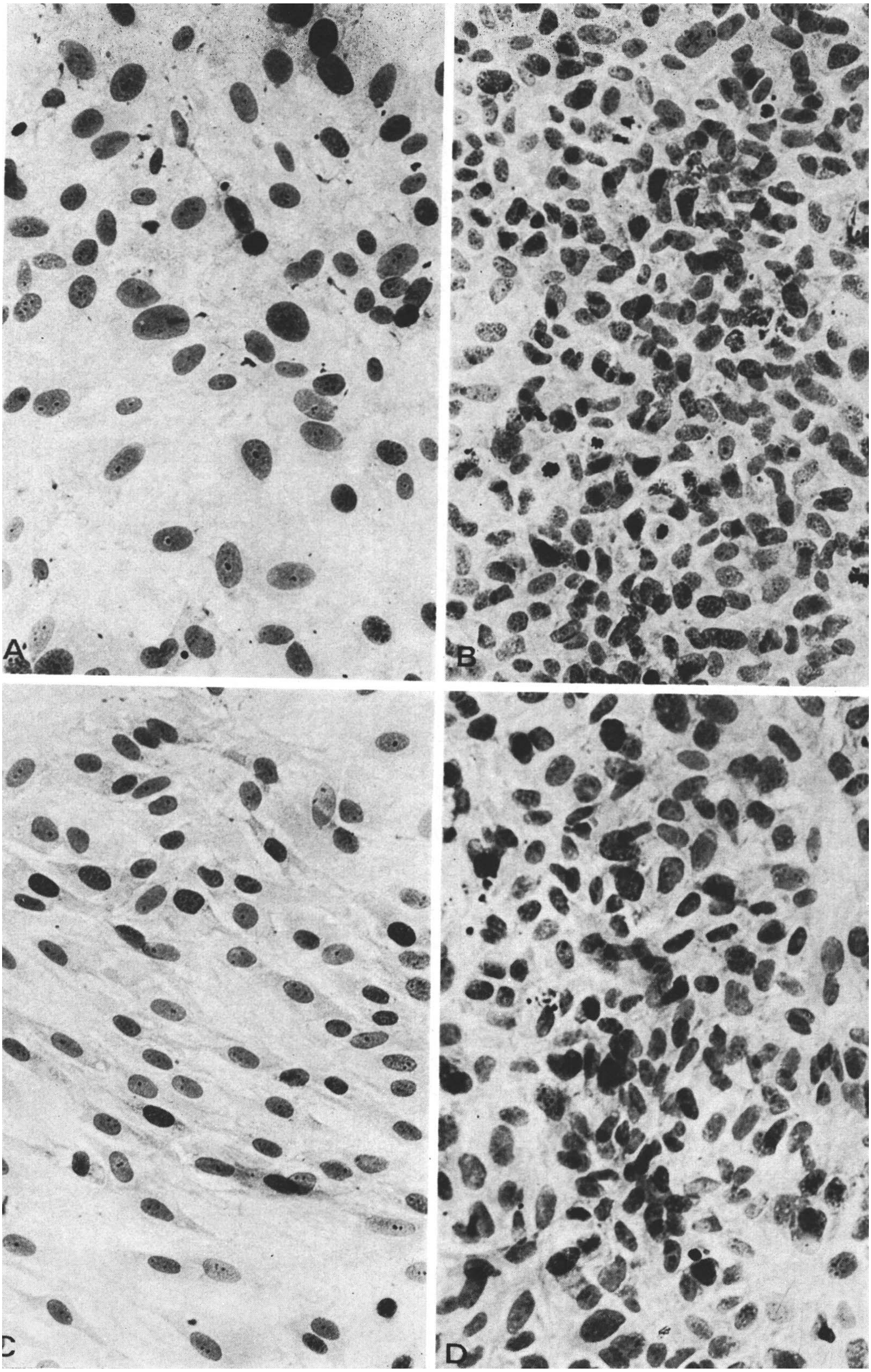
time = ca. 6 days), but subsequently grew at a rate similar to that of GE-1 and GE-2 cells.

Temperature optimum. The GE-1 cells exhibited a maximum growth rate at 29–31°, with a sharp reduction in growth rate at temperatures above and below this range (Fig. 3). This temperature optimum is similar to that of the adult *Gekko* cell line GL-1 (17).

pH optimum. The growth rate of GE-1 cells was also compared with that of GL-1 cells as a function of pH in the range of 6.6–8.5. Medium maintained in a pH range of 6.9–7.7 supported optimal growth of GE-1 cells (Fig. 4), while growth was severely retarded at pH $> 7.9 < 6.8$. The effect of pH on growth of GL-1 cells was similar. The attachment of GE-1 cells was more pH-dependent than was attachment of GL-1 cells.

Virus susceptibility of GE-1 cells. GE-1 cells were susceptible to CPE (observed during at least two serial virus passages) induced by herpes simplex, vaccinia, vesicular stomatitis, Sindbis, Newcastle disease, and frog polyhedral viruses. Efficient production of CPE by VSV required that cells be infected at a m.o.i. of $\leqq 1.0$. Rabies virus caused an abortive infection (see below). Following infection with polyoma virus at a m.o.i. of 1.0, T antigen formation developed in from 0.1 to 1.0% of cells. T antigen persisted at this level for 25 infected cell passages at 30°, but morphologic transformation was not observed. SV40 caused cell transformation (see below). GE-1 cells exposed to viper "C-type" virus (18) or murine sarcoma virus (Maloney) and subsequently subcultured 20 times at 30° exhibited no morphological alteration.

Comparison of rabies virus replication in gecko cell lines. Cultures of cell lines GE-1, GE-2, WGT-1, and GL-1 were infected with rabies virus at a m.o.i. of 30–100 and incubated at 33°. In all cell lines, immunofluorescent rabies antigen was detected in from 19 to 74% of the cells on the first day after infection. However, the incidence of infected cells subsequently decreased, and little or no released virus production was detected in cell supernatant fluids. Serial subculture of rabies virus-infected cells was not readily accomplished at 33°; serial cell subcultivation at 30° was accompanied by progressive disappearance of rabies virus antigen within three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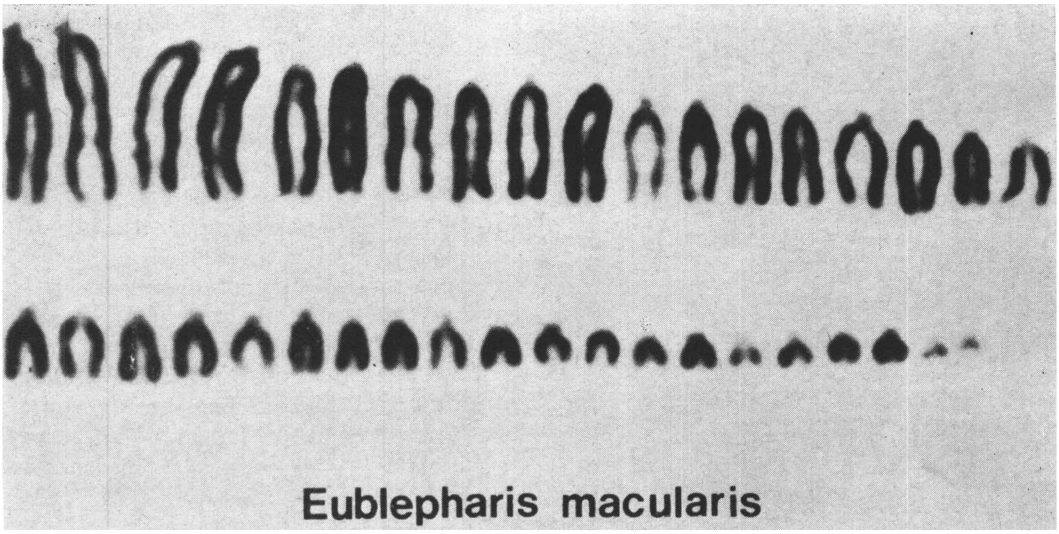

FIG. 2. The diploid karyotype of *Eublepharis macularis*. The 2N chromosome number is 38. All chromosomes are acrocentric, forming a graduated series according to size.

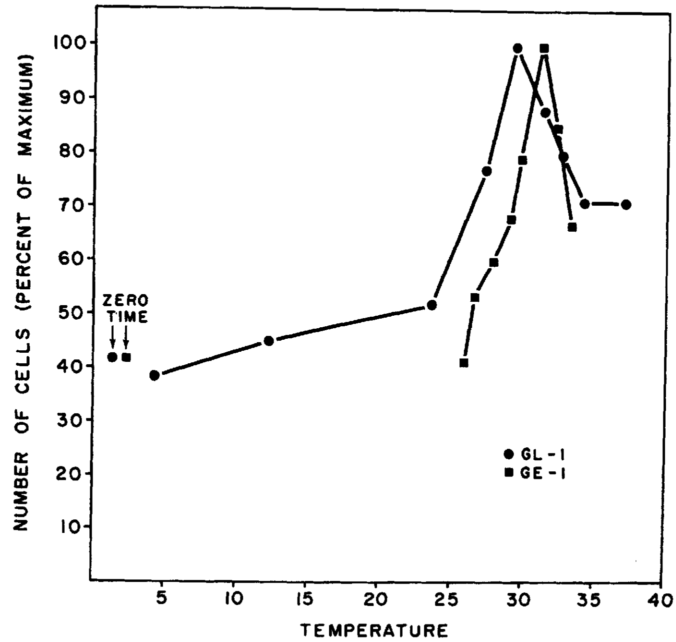

FIG. 3. Effect of temperature on the growth rate of *Eublepharis* GE-1 and *Gekko* GL-1 lizard cells. Replicate 25 cm^2 plastic culture flasks were seeded with 8.0×10^4 cells each, incubated at 30° for 24 hr to allow for uniform attachment, and then placed at different positions in a temperature gradient incubator. When the cell growth became confluent in the most rapidly growing culture, the cells in all cultures were counted.

FIG. 1. *Eublepharis* cells *in vitro*. All cells stained by May-Greenwald-Giemsa technique and photographed at 78×. *A*—Cell line GE-1, 59th passage. Epithelial-like cells exhibiting contact inhibition. *B*—Cell line GE-1, 60th passage, transformed by SV40. Dense, multilayered growth of slightly smaller cells. Many mitotic figures are visible. *C*—Cell line WGT-1, 42nd passage. Fibroblast-like cells exhibiting contact inhibition. *D*—Cell line WGT-1, 42nd passage, transformed by SV40. Dense multilayered growth of smaller, more epithelial-like cells.

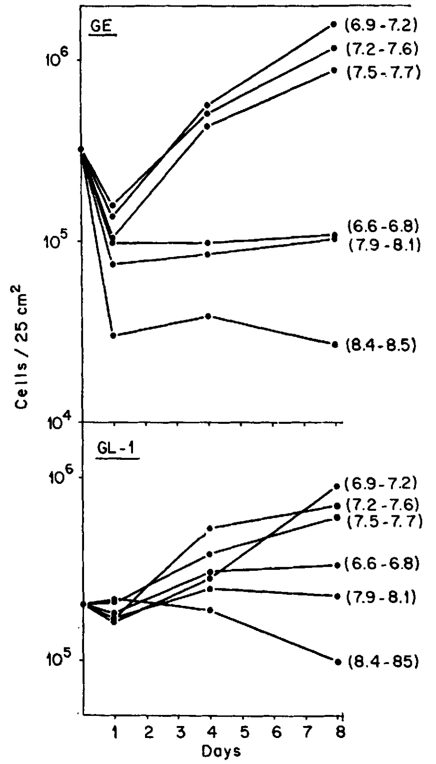

FIG. 4. Effect of pH of culture medium on growth of *Eublepharis* GE-1 cells and *Gekko* GL-1 cells. Replicate cell cultures were seeded with identical numbers of cells and fed with medium buffered at differing pH with organic ions. Medium was changed on days 2 and 5 after cell plating.

passages. Apparent abortive infection of this nature has not previously been reported with rabies virus in cell culture (Wiktor, T. J. and Clark, H F., manuscript in preparation).

Growth rate studies of Sindbis virus in reptile and BHK/21 cells. The single step growth curve of Sindbis virus, following infection at a m.o.i. of 10.0 and incubation at 33°, was determined for cells of three *Eublepharis* cell lines, the lizard cell lines GL-1 and IgH-2, and the mammalian cell line BHK/21 (Fig. 5). The latent period in BHK/21 cells was found to be less than 4 hr and the maximum titer of released virus was produced within 10 hr. The latent period in the established reptile lines GL-1 and IgH-2 was also less than 4 hr, but the subsequent logarithmic phase of virus replication proceeded at a slower rate; the peak titer was not reached until 15 hr. The viral growth curves in adult and embryonic *Eublepharis* cell lines were almost identical; the latent period was approximately 8 hr and the maximum titer was not achieved until 48 hr. The final yields of released virus obtained from all cell lines were similar.

SV40 transformation. GE-1, GE-2, and WGT-1 cell cultures were infected with SV40 at a m.o.i. of 20–100, maintained at 33° for 7 days, and subsequently subcultivated at 6–10 day intervals at 30°. In two replicate experiments, morphological transformation of GE-1 cells was first detected at the fourth (40 days) and thirteenth (80 days) passages postinfection, respectively. SV40-infected GE-2 and WGT-1 cultures both first exhibited morpho-

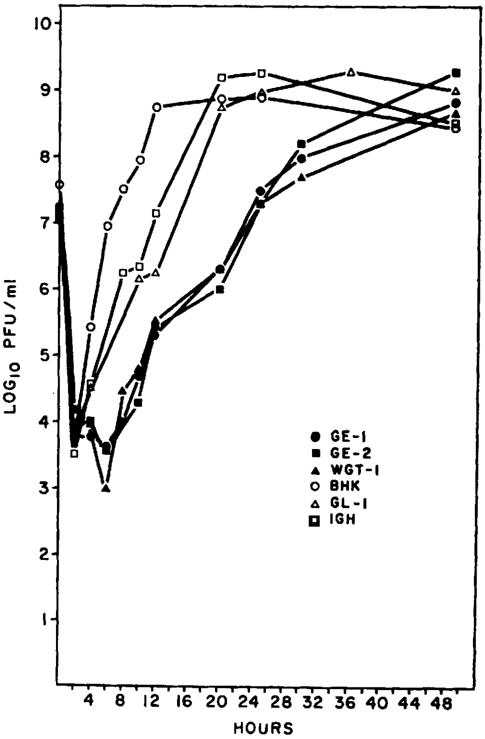

FIG. 5. Single-step growth curves of identically infected cultures of *Eublepharis* embryo-(GE-1 and GE-2) and adult-(WGT-1) origin cell lines, of adult lizard cell lines of *Iguana* (IgH-2) and *Gekko* (GL-1) origin, and of a hamster-(BHK/21) origin cell line. All cultures were infected at a virus multiplicity of 10.0 and incubated at 33°. Cell supernatant fluids were harvested at the times indicated and assayed by plaque method in BHK/21 cell cultures.

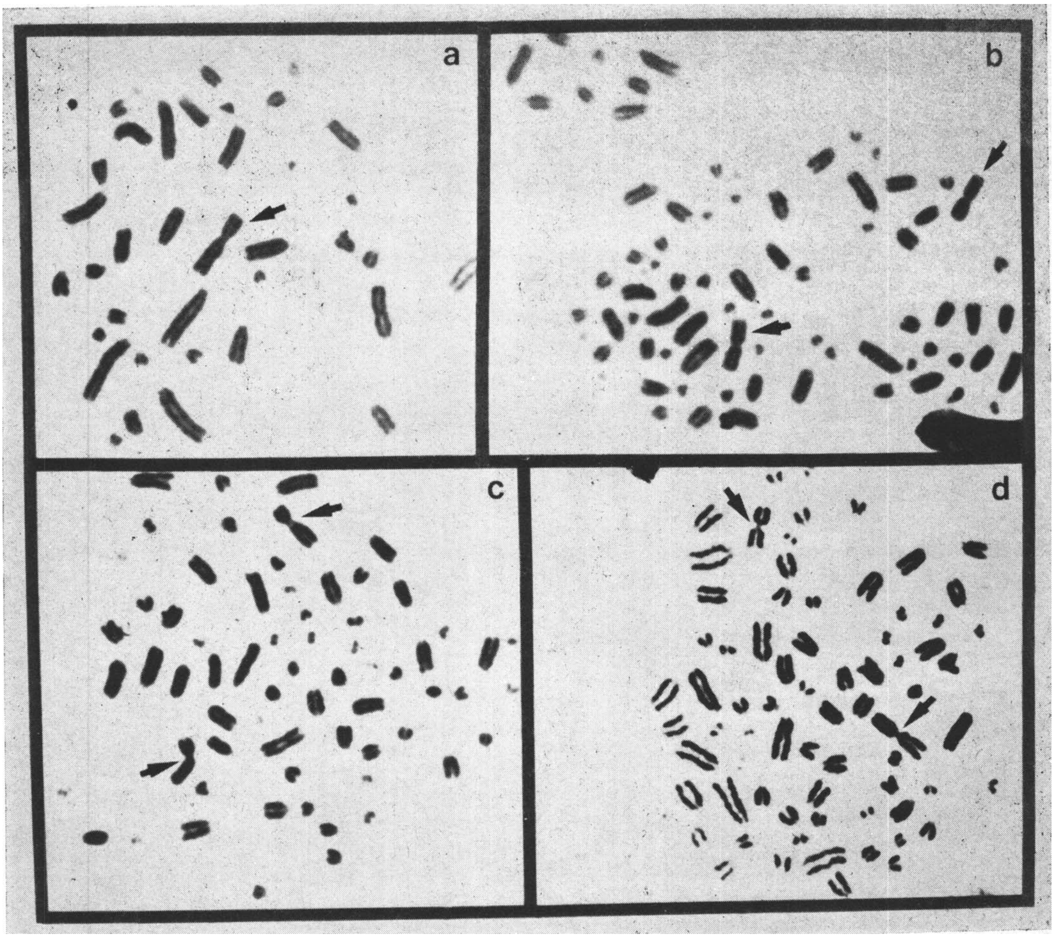

FIG. 6. Metaphase chromosomes of SV40-infected GE-1 cell, 16th infected passage. *A*—Cell with 37 chromosomes with submetacentric marker chromosome (arrow). *B*—Polyploid cell with two identical metacentric marker chromosomes (arrows). *C*—Polyploid cell with two identical submetacentric marker chromosomes (arrows). *D*—Polyploid cell with two dissimilar metacentric marker chromosomes (arrows).

logical transformation at the ninth postinfection passage (60–70 days).

The transformed cells (Fig. 1B and D) became epithelial-like and exhibited an increased nuclear cytoplasmic ratio, with pleomorphic nuclei, some of which were fragmented, and nucleoli that were usually increased in size and number. The incidence of mitotic figures was elevated, and many were abnormal. Also, as is characteristic of SV40-transformed mammalian cells (19) there was irregular basophilia in the cytoplasm. The mean cell volume of the nontransformed GE-1 cells was 2700 μm^3; that of the transformed cells was 1600 μm^3.

The GE-1 cell karyotype (Fig. 2) was analyzed both before and after morphologic transformation. Before morphological transformation (infected passage 10), the only chromosomal change was an increase in polyploidy (14% 4N cells). After morphological transformation (passage 16), the modal number of chromosomes had shifted to 37 and both the chromosome breakage rate (16%) and the level of polyploidy (30%) had increased. The reduction in chromosome number was accompanied by the emergence of metacentric and submetacentric elements (Fig. 6A and B), probably derived via Robertsonian translocations between normal acrocentric

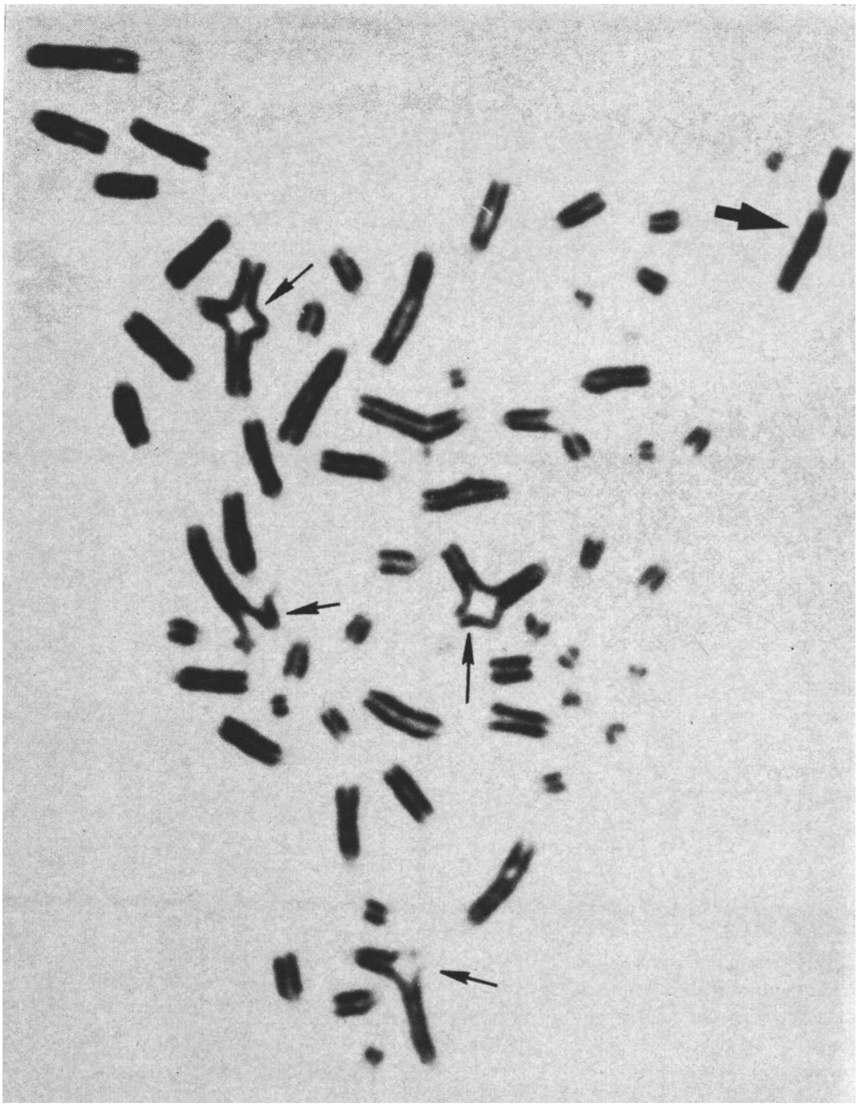

FIG. 7. Metaphase from SV40-infected GE-1 cells at virus passage 24. Arrows mark tri- and quadriradial configurations indicative of chromosome breaks and chromatid exchanges. Large arrow indicates submetacentric marker chromosome.

chromosomes, thereby reducing the chromosome number. In tetraploid cells, two such metacentric markers were present. In some cells both chromosomes were of similar size and morphology (Fig. 6B and C) indicating either endoreduplication or fusion of two cells with like markers. The metacentric markers in Fig. 6A and C are different, being much more nearly metacentric. Figure 6D shows a tetraploid cell with two obviously dissimilar metacentric markers which arose through the fusion of two cells with different markers. The morphological variation in the metacentric markers strongly suggests that multiple fusion events occurred between different acrocentric chromosomes. Forty-two cells (84%) had at least one metacentric marker at this passage level.

By infected passage 24, a modal chromosome number of 65 (and a range of 38–96) was observed. Virtually the entire cell population was tetraploid at this passage. Chromosomal damage was readily apparent in the many breaks and tri- and quadriradial configurations (Fig. 7), the latter indicative of chromatid exchanges. These chromosomal changes are similar to those reported when adult gecko cells were transformed by SV40 (9).

In transformed GE-1 and WGT-1 cells the growth rate was not remarkably different from that of the nontransformed cells, but the saturation density was increased (Fig. 8) and acidification of the medium occurred at an accelerated rate. Contact inhibition of cell movement was incomplete. The transformed cells were readily propagated at the supraoptimal temperature of 35°, at which temperature control cells were not readily grown. The absolute plating efficiency of SV40-infected GE-1 cells was markedly enhanced (13% at the 11th infected passage, prior to the appearance of morphological transformation, as compared with 0.2% in nontransformed cells [12th passage]).

SV40 T antigen (Table II) was present in *Eublepharis* cells immediately after infection. A sharp increase in the percentage of T antigen-positive cells was noted at the time of morphologic transformation, as has been observed in mammalian cells (20). *Gekko* (GL-1) cells (previously reported to be susceptible to SV40-induced transformation), infected in parallel, did not exhibit transformation during a time period leading to expression of transformation in each of four *Eublepharis* cell types.

Attempts to isolate SV40 from the supernatant fluids of transformed GE-1 cell cultures at virus passage levels 17 and 23 were unsuccessful. SV40 was successfully recovered from transformed GE-1 cells at infected passage 22 by cocultivation with CV-1 cells at 35°.

SV40-transformed GE-1 cells could also be distinguished from normal cells by their capacity to form colonies when suspended in soft agarose at 30°. A colony-forming efficiency of approximately 4% was observed with cells tested in the 41st infected passage. Control cells formed < 1% colonies under similar conditions.

Transformed GE-1 cells were tested for the presence of transplantation antigen by assay of their ability to protect hamsters against SV40-induced tumor formations. Newborn

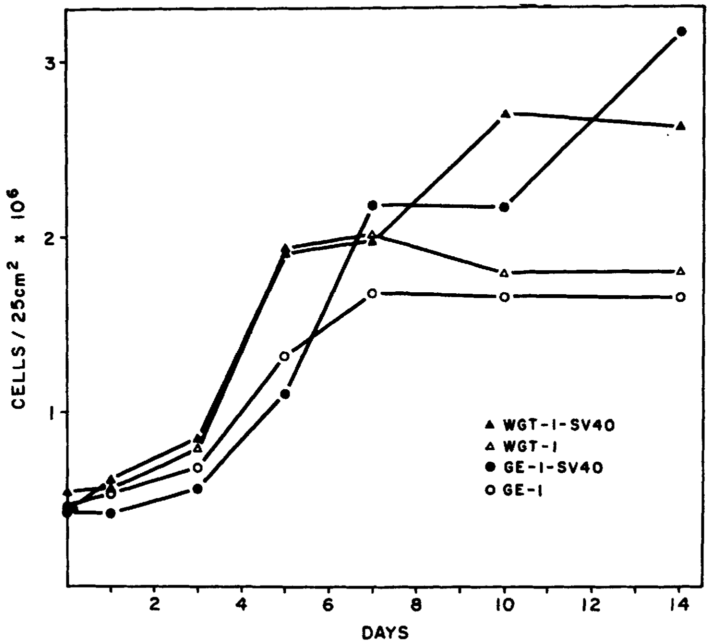

FIG. 8. Growth rates of adult-(WGT) and embryo-(GE) origin *Eublepharis* cell lines before and after transformation by SV40. 4.0 to 5.0 × 10^5 cells of each cell type were plated in 25 cm^2 plastic flasks, fed with 5.0 ml of BME-FCS 10 (with *no* refeeding), and incubated at 30°. Transformed adult and embryo cells were tested at the 14th and 22nd passage, respectively, after infection.

animals were inoculated with $10^{7.0}$ PFU of SV40 and immunized 44 days later with a dose of 1.6×10^7 transformed GE-1 cells. At 177 days after virus inoculation, seven of nine control hamsters had tumors; the tumor incidence in immunized animals was five of nine.

Discussion. There is widespread opinion that mammalian and avian embryonic cells are more readily cultivated, and are more sensitive to many virus infections, than are adult cells, but remarkably few systematic comparisons of these characters have been reported. It has been reported by Black and Rowe (21) that there was little difference in susceptibility to SV40-induced transformation between adult and embryonic hamster cell lines, although only newborn hamsters are susceptible to the SV40 tumorigenic effect *in vivo* (20, 22).

We have found that cell lines could readily be established from tissues of an embryonic lizard, more easily than from adult tissues of the same species. Previous attempts to cultivate embryonic reptilian cells have not led to establishment of cell lines (23, 24). The tail tissues of adult lizards that have a regenerative capacity appear to be the tissues of choice for cell culture. The donor of the tail tissue can be used in subsequent experimentation with its own cells.

Few differences were found between the properties of cells cultured from tissues of the embryonic or adult leopard gecko, and those of previously described reptile cell lines from several other species (25). Similarities include the optimal medium for growth, cell morphology, growth rate after adaptation to culture, optimal temperature, virus susceptibility, and capacity to be transformed by SV40. It is interesting to note that the pH optimum of 6.9–7.2 determined for both GE-1 and GL-1 reptile cells is slightly lower than the optimal pH ranges reported for a majority of mammalian cell types tested by similar methods (26).

Quantitative studies of susceptibility to infection with an arbovirus, Sindbis virus, as measured by comparison of single-step virus growth curves, suggested that host cell species plays a more important role than does the age of the cell donor. Sindbis virus growth curves obtained in adult and embryonic *Eublepharis* cell lines were indistinguishable, yet both differed markedly from those obtained in

TABLE II. Percentage of Reptile Cells Exhibiting SV40 T Antigen.[a]

Cell line	Cell passage after infection												
	1	3	4	5	6	7	8	9	10	11	12	13	22
G-1													
Expt 1		<1		<1							25	60[b]	100
Expt 2	41	34	79[b]		99		97		99				100
GE-2	44	10	5	0	11		72	[b]	94		84		100
WGT-1	19	44	0	17		39		57[b]		91			100
GL-1	<1	0	0		4		10						2

[a] Cell cultures were infected in parallel with SV40 at a virus multiplicity of infection of 20–100, maintained at 33° for 7 days after infection, and then subcultivated at 6–10 days intervals with incubation at 30°. The incidence of cells expressing T antigen was determined by immunofluorescent technique.

[b] Onset of morphologically transformed appearance.

cell lines derived from two other species of reptiles, which in turn deviated from a virus growth curve obtained with mammalian BHK/21 cells.

The repeated transformation of leopard gecko cells extends our previous findings, with cells of another species (*Gekko gecko*) of the same family, that reptilian cells can be transformed *in vitro* by the mammalian virus SV40 (6). Another papovavirus, polyoma virus, did not cause transformation. Ponten (19) has reported that polyoma virus is best able to transform cells that are genetically unstable, while SV40 can transform both stable and nonstable cells. The *Eublepharis* cell lines were euploid when infected. A low level of infection of GE-1 cells with polyoma was indicated by persistence of T antigen for 25 serial cell passages at 30°; this is apparently the first report of infection of poikilothermic cells with polyoma virus.

When compared to our previous report of the hyperdiploid reptile *Gekko* cell transformation, the SV40-induced changes in the *Eublepharis* karyotype were more readily characterized because *Eublepharis* cell lines were diploid. The results of this analysis are similar to those reported for mammalian cells (27). In contrast to *Gekko* GL-1 cells, in which the immediate morphologic response to transformation was a restriction to monolayer formation, followed later by unrestrained growth, the immediate response of transformed *Eublepharis* cells was a change from restricted to unrestricted growth. SV40-transformed *Eublepharis* cells also differ from transformed GL-1 cells in their very limited expression of SV40 transplantation antigen.

SV40-infected cells were subcultivated at about weekly intervals until after an interval of 6–11 wk at 30° morphologic transformation occurred. In phase II human embryonic fibroblasts, transformation commences after 6–16 wk at 37°, and transformation in hamster cells occurred after about 3–4 wk at 37° (19). Thus, *Eublepharis* cells incubated at 30° appear to transform at a rate similar to that observed in many mammalian cell types incubated at 37°.

The *in vivo* capacity of these transformed cells to induce tumors is now being investigated. In a preliminary test, two allogenic animals were injected subcutaneously with SV40-transformed GE-1 cells, and after 4 mo, no growth has taken place. It will be interesting to determine whether these lizards which can regenerate their tails, representing the highest group of vertebrates retaining a considerable measure of regenerative ability, can succumb to *in vivo* tumorigenesis. Such a system would permit a detailed study of the interaction of regeneration, differentiation, and carcinogenesis.

Summary. Cell lines were established from trypsin-dispersed or minced and explanted tissues of whole embryo or adult tail tissues of the leopard gecko, *Eublepharis macularis.* Embryo cells grew more rapidly at early passage levels, but an adult cell line exhibited a growth rate similar to that of embryonic cells (mean cell population doubling time = ca. 3.5 days) after adaptation to *in vitro* growth for 12 passages (14 wk). Embryo-origin cells were predominantly epithelial-like, whereas adult tail-origin cell lines were fibroblast-like.

Adult- and embryo-origin cell lines could not be distinguished on the basis of temperature optimum (29–31°), medium pH requirements (pH 6.9–7.7), karyotypic changes (all passage levels tested [to number 25] were euploid), or virus susceptibility. Adult and embryonic leopard gecko cells supported cytopathic infection by vaccinia, herpes simplex, frog polyhedral, Newcastle disease, Sindbis and vesicular stomatitis viruses. Single step growth curves of Sindbis virus were identical in adult and embryonic *Eublepharis* cells incubated at 33° but differed from curves determined in other reptilian and mammalian cell types. Both adult and embryonic cells supported abortive rabies virus infection.

SV40 caused transformation at a similar rate of adult and embryonic cells incubated at 30°. Transformed cells exhibited 100% T antigen expression, morphologic and karyotypic alterations, and increased cell saturation density, absolute plating efficiency, and efficiency of colony formation in soft agarose. Tumor-specific transplantation antigen induction was not evident. Adult gecko tail-origin fibroblasts transformed by SV40 *in vitro* have not caused tumors to date when reinoculated into the allogenic host animal. Gecko cell cultures infected with polyoma virus at

30° exhibited $\leqq 1\%$ T antigen expression that persisted for 25 cell passages, but did not lead to transformation.

This investigation was supported in part by U.S. Public Health Service Research Grant AI-09706 from the National Institute of Allergy and Infectious Diseases, RR-05540 from the Division of Research Resources and Training Grant CA-05163 from the National Cancer Institute.

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Received January 28, 1974. P.S.E.B.M. 1974, Vol. 146.